A PRELIMINARY STUDY ON HISTOLOGICAL EFFECT OF SALTED FISH (*OPHIOCEPHALUS STRIATA*) IN ALBINO RATS

Geertruida Sihombing; Cornelis Adimunca; Risnawati; Trisulo; Iwan T. Budiarso*

ABSTRACT

Tiga puluh ekor tikus putih umur 28 hari dibagi menjadi 3 kelompok. Setiap kelompok terdiri atas 10 ekor. Grup I diberi makan pakan non-protein, Grup II diberi pakan 10% susu skim dan Grup III diberi pakan 10% protein ikan asin. Makanan dan minuman diberikan secara ad. libitum. Setiap ekor sebelum percobaan dimulai dan 2 kali seminggu selama masa observasi ditimbang berat badannya. Pada hari ke 28, setiap tikus diukur protein efficiency rationya. Percobaan ini diamati selama 4 bulan penuh. Dalam masa observasi, ditemukan 3 ekor tikus Grup III mati dan 2 ekor tampak kurus serta bulunya kasar dan tidak berkilau. Pada otopsi dari tikus yang mati ditemukan mukosa bagian posterior orofaring berwarna keputihan dan permukaannya bergranuler. Pada pemeriksaan mikroskopik jaringan ini menunjukkan reaksi radang kronis dan disertai dengan hiperplasi dan hiperkeratosis lapisan mukosa. Lapisan epitel beberapa asinus kelenjar ludah mengalami skuamus metaplasia dan ada pula yang disertai mutiara keratin. Ada beberapa lagi asinus yang mengalami delatasi dan lumennya dilapisi epitel gepeng. Alat-alat tubuh lain tidak mengalami perubahan yang nyata.

INTRODUCTION

Human exposure to carcinogens have been reported in various articles from many countries. They are of endogenous of exogenous origin. Foods such as sausages, frankfurters, fish products contain nitrite or nitrate (either naturally or by addition during processing) and primary or secondary amines. These substances are known to be precursors of N–nitroso compounds. Many studies have confirmed that some nitroso compounds can occur during cooking but also within the stomach after ingestion of precursor materials in food or following administration of drugs. All the findings have led to the reassesment of the use of nitrite as a food preservative.[1] Nitroso compounds such as nitrosamines, are carcinogenic in a wide range of animal species, and their formation from a variety of precursors in the bodies of animals have been demonstrated, which may also occur in humans. Human diets may contain a great variety of carcinogens, the nature of which may theoretically be of exogenous origin.

* Non-Communicable Diseases Research Centre, NIHRD.

As an important source of good animal protein, fish products of many varieties are consumed in Indonesia as well as in other ASEAN Countries. They are well-known as salted dried fish, fermented and also smoked fish products. Salted dried fish are widely consumed throughout Indonesia especially in the villages where fresh fish are rarely found. Taking into consideration, the fact might contain N-nitroso compounds, it is appropriate to conduct an animal feeding experiment using albino rats for obtaining a deeper insight in this matter.

MATERIALS AND METHODS

Fish

Dried salted fish of *OPHIOCEPHALUS STRIATA* (Ikan gabus) was chosen as sample since this kid of fish is widely consumed by the Indonesian people. Fish sample was bought from a supplier in the Jakarta market. In the laboratory, the sample was washed under tap water to get rid of kitchen salt, and dried in the oven at 60 degree centigrade for about 24 hours. The flesh was then separated, grounded into powder and used for the ingredient of the experimental diet.

Diets

The experimental diets were prepared at the recommended level of 10% protein and 10% fat. A positive control diet of fresh skim milk powder obtained from New Zealand also at the level of 10% protein was used.

Protein free diet as a negative control diet was also incorporated. The composition of the diets are shown in Table 1.

Table 1 : Composition of foddstuffs in prepared diets

	Non Protein	Skim milk 10%	Dried salted fish 10%	Stock diet
Fat	100.00	100.00	92.66	42.80
Starch	720.00	547.39	633.92	285.80
Glucose	148.50	50.00	50.00	50.00
Salt Mix	20.00	20.00	20.00	20.00
Cellu Flour	11.50	20.00	20.00	20.00
Vitamin Mix	+	+	+	+
Skim milk	–	262.61	–	–
Dried salted fish	–	–	183.42	–
Stock diet	–	–	–	581.40

Chemical analysis

The proximate composition of the diets were determined according to the methods of Analysis of Association of Official Analytical Chemicals (AOAC).[2]

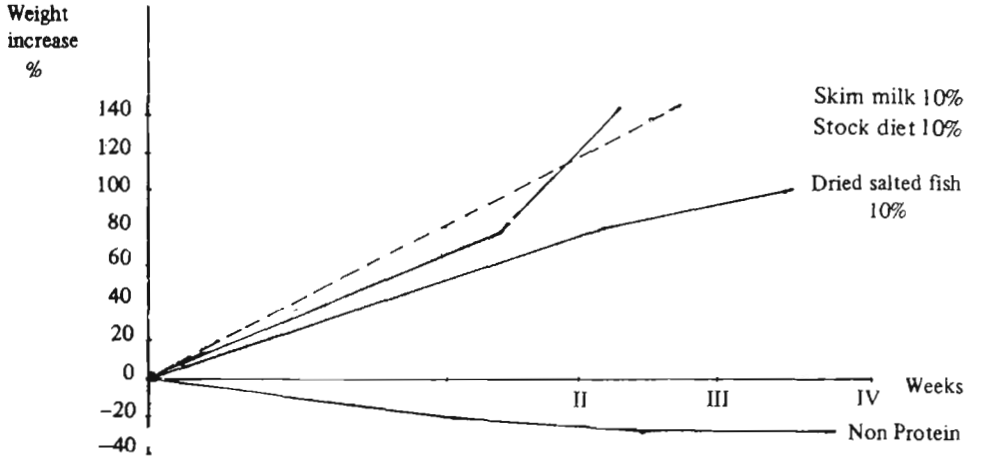
Rat Feeding experiments

For the animal feeding experiments, young weanling 28 day-old albino rats were obtained from our own Nutrition Research Unit colony. In the effort to see the toxic effect of the salted fish, the Protein Efficiency Ratio (PER) value of the experimental diet was first determined. This value was taken into consideration in planning the length of period of the feeding experiment.

For PER determination, thirty weanling of twenty eight day old male rats were used. A randomized blocks design was used in which blocks represent variations in initial weight. The rats in each block were also randomized for diet and cage. They were placed in individual metal-wired cages with paper as litter absorbant. They were divided in three groups which consisted of 10 animals each. Goup I was fed on non-proteint diet. Group II was fed on 10% skim milk diet, and Group III was fed on the experimental diet containing 10% dried-salted-fish protein. The animals were observed for a 4 week period. All animals were given food and drinking water *ad. libitum*. During the observation period, each rat was weighted individually as well as the individual food consumption which was recorded twice at weekly intervals. From the amount of food consumed, and total body weight gain, the individual Protein Efficiency Ratio (PER) values were calculated as gram gain of each gram protein eaten.[3]

After four weeks of PER determination, the feeding experiment was extended for about four month in order to see the possibility of toxic effect. At the end of the feeding period, all animals were sacrified for necropsy to see the lesions.

Figure 1 : Body weight gain of rats fed on dried salted fish, skim milk and stockdiet.



RESULTS AND DISCUSSION

The approximate composition of the ingredients used in the diets are shown in Table 2. The protein contents per 100 grams were found 54.52 grams for dried salted fish, whole for skim milk and stock-diet were 38.08 gram and 17.26 gram respectively. The high protein content of dried salted fish gave the impression that this product could be expected to be a good protein source for human consumption. However, animal feeding experiments on Protein Efficiency Ratio (PER) examination showed a lower value in animals fed on dried salted fish i.e. 2.27 $\pm$ 0.52 as compared to skim milk diet and stock diet i.e. 2.92 $\pm$ 0.10 and 2.71 $\pm$ 0.14 respectively. Another comparison to show this low PER value could be presented as the result of another PER experiment using rat fed on fish mixed with cassava and soy.[4] The dried fish sample was prepared from fresh fish by steaming, drying in the oven at 60 C and grounded to powder. The PER value at 10% protein level was found to be 2.70.

Table 2 : Proximate analysis of Food Items used as diets (g per 100 g).

Food Item	Mois-ture g	Pro-tein g	Fat g	Carbo hydrate by diff g	Ash
1. Rice (Nx5.95)	14.58	8.82	0.28	75.95	0.37
2. Peanut (Nx5.48)	7.30	23.40	44.00	21.78	3.52
3. Soy-bean (Nx5.71)	9.29	37.10	19.95	29.16	4.50
4a. Dried salted fish (Nx6.25)	3.31	54.52	4.00	8.76	29.41
4b. Dried salted fish (Nx6.25) 10%	–	10.22	10.00	–	–
5a. Skim milk (Nx6.38)	2.56	38.08	–	52.11	7.25
5b. Skim milk 10% (Nx6.38)	–	10.46	10.00	–	–
6. Stock diet (Nx5.95)	9.62	17.26	9.83	59.92	3.37

The PER values presented above showed that the digestable and absorbable protein value may be destroyed partially during the salting-drying process. The

most probable cause might be due to the delayed and prolonged drying period, for example because of unpredictable bad weather leading to the deterioration of some essential amino-acids. Another cause may be attributed to some kind of denaturation process during the salting and drying period. It is known that some fishermen use chemical preservatives such as nitrite or nitrate or pesticides.[5,6] The presence of nitrite and nitrate either intentionally added from kitchen salt, or other chemical contaminants as residues of pesticides might cause metabolic and absorption interference in the gut. All of the above mentioned factors were incriminated as underlying cause of the decrease in bio-availability.

Clinical observation showed that the body weight gain in the salted fish group was significantly lower than those of skim and stock diet group (Figure 2). This finding was in line with the PER value showed by the experimental animals (Table 3). Other clinical signs were roughened hair coat, especially in two animals found dead. In salted fish group one rate each died at week 12, 14 and 15. The other seven animals were still alive at the end of the experimental period.

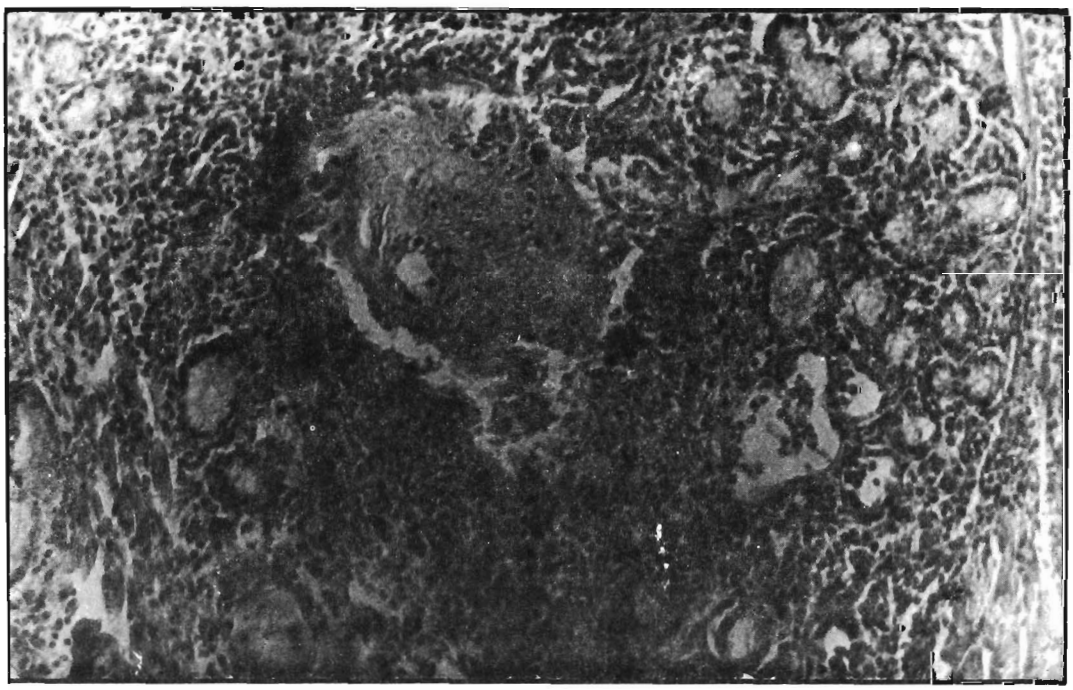


Figure 2. Posterior oropharyngeal area of rat fed 10% protein salted fish. Note the hyperplastic and hyperkeratotic changes of the lining epithelial mucosa. Two acinus glands underwent hyperkeratitic, hyperplastic and cystic changes. The surrounding connective tissue was infiltrated by inflammatory cells. 40X, H & E.

Table 3 : Summary of Results of Protein Efficiency Ratio Determination per rat by weeks of : Skim milk 10%, Stock diet 10%, Dried salted fish 10%.

Experimental Diet	No. of rats n	Protein Content of XP-diet g%	Avg. wght of Start	Avg. Cumulative wght. Increase in % of Starting weight at End of week no.				Total Weight Gain	Avg. Total Food-Intake per rat by week during week no.				Total Food-Intake	Total Protein Intake	PER ± SD
				I %	II %	III %	IV %	g	I g	II g	III g	IV g	g	g	g
Protein-free	10	0	45,65	-14,2	-20,5	-27,2	-31,0	-14,10	19,2	16,9	14,8	15,5	66,4	0	0
Skim Milk	10	10,16 (Nx6,38)	45,65	30,9	58,3	93,9	134,2	61,26	44,5	53,8	54,7	53,5	206,5	20,98	2,92+0,10
Stockdiet 10%	10	10,59 (Nx5,95)	45,65	31,8	64,9	94,9	130,1	59,40	45,0	52,7	54,1	55,5	207,3	21,97	2.71+0,14
Dried salted	10	10,22 (Nx6,25)	45,65	22,7	47,9	74,2	93,9	42,9	40,3	46,9	49,5	47,7	184,4	18,85	2,27+0,52

At necropsy, two out of the three dead rats showed abnormal appearance around the mucosa of the posterior oropharyngeal area. These were whitish in colour and granular in appearance. Histological examination on these two revealed inflammatory reactions. One rat showed more intense reaction than the other, but basically they were similar. The epithelial mucosal lining thickened and showed the presence of a hyperkeratotic process. One rat also showed keratin-pearls both in the mucosal layer as well as in the squamous metaplastic salivary glands. The lumen of some acinus gland were dilated and lined by flattened epithelial cells. Others also showed keratine pearls in the squamous metaplastic salivary gland (Fig. 2 and 3).

The internal organs such as the hear, lungs liver, spleen, kidness and gastro-intestinal tract of the salted fish group as well as the skim milk and stock diet group no pathological lesions were found. The above mentioned findings indicate that several factors may be involved such as the high content of sodium chloride which could act as an irritant and cause atrophy and metaplasia in the mucosal layer, followed by bacterial over growth and nitrite formation. All of these processes can lead to the subsequent synthesis of nitroso compounds known to be carcinogenic. Other factors which may contribute substantially to these inflammatory reactions are the residual content of chemical preservatives and pesticides.

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

From the preliminary animal feeding experiment using albino rats fed on dried-salted-fish diet the following conclussions can be made :

1. The Protein Efficiensy Ratio (PER) value of the dried-salted-fish diet was lower than those of skim milk and stockdiet.
2. The ten percent salted fish diet

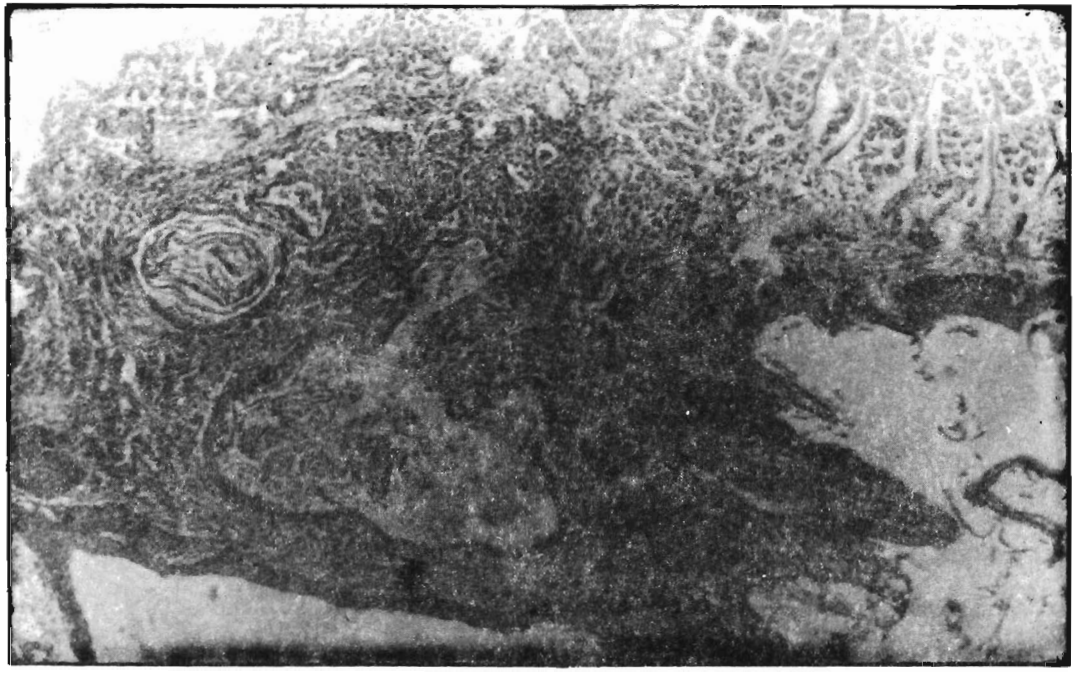


Figure 3. Salivary glands in the posterior oropharyngeal area of rat fed 10% protein salted fish. Note the acinus gland underwent squamous metaplasia and the surrounding tissue was heavilly infiltrated by inflammatory cells. 40X, H & E.

caused 20% inflammatory reactions in the posterior oropharyngeal region of the experimental animals.

3. The nutritional value of the dried-salted-fish was decreasing, perhaps due to the deterioration and denaturation of some essential amino acids during the salting and drying process.
4. The toxic effects of this salted fish may be due to the irritatating effect of sodium chloride, contamination of nitrite, nitrate, residue of preservatives or pesticides or the mixture of them.
5. Since dried-salted-fish is an important animal protein source in Indonesia, the safety of the product should always be taken into consideration.
6. Since dried-salted-fish is much consumed by the people at large, therefore, other than *Ophiocephalus striata* (ikan gabus), is warranted to be investigated for the safety of human consumption.
7. Information on the appropriate method of salting-drying fish should be dissiminated to salted fish producers throughout the country, in order that good quality and safe products are provided for the consumers.
8. Data obtained from this investigation can be of an important source of information for the health workers as well as for decission-makers.

REFERENCES

1. Crosby, N.T. (1985) : Nitrosamines: A review of their chemistry, biological properties, and occurence in the environment. In Book, Chemical Analysis of Inorganic Constituents of Water. John C. Van Loon, CRC Press. Florida, 185, p. 131.
2. Horwitz, W. (1975) Methods of Analysis of the Association of Official Chemists, Twelfth Edition, 1975.
3. Civo Institutes TNO : Evaluation of Protein Quality in Rats, Civo-TNO (41), 82-03-12/Fdv-JB. (no year publication).
4. Diponegoro Nutrition Research Unit (1980), National Institute of Health Research and Development, Ministry of Health: Results of rat feeding experiments practical laboratory work, medical students Atmajaya University, 1980.
5. Djayusmantoko (1983) : Masalah dan pembuatan ikan asin pada musim panas dan musim hujan di daerah Muara Angke, Jakarta Utara. Skripsi Akademi Gizi, Departemen Kesehatan R.I., Jakarta 1983, Hal. 45-47.
6. Indonesian Consumer Organization : Personel Communication, 1989.